Rapid Protein Identification Using a Microscale Electrospray LC/MS System on an Ion Trap Mass Spectrometer

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A methodology has been developed for the rapid identification of gel separated proteins. Following in gel protein digestion with trypsin, the resulting peptide mixture is analyzed by on-line liquid chromatography, electrospray mass spectrometry (LC/MS). The mass spectral data containing either accurate mass values or sequence specific fragment ion information is then matched to a database of known protein sequences. Key features of the LC/MS system are the use of a novel integrated, microscale LC column–electrospray interface and variable flow solvent delivery to optimize the efficiency of sample loading and gradient elution. With these enhancements, only 10 min is required to analyze each sample. The method is routine for sample amounts ranging from 50 to 500 fmol. The analysis parameters for the ion trap mass spectrometer have to be carefully adjusted in order to keep pace with the rapidly eluting LC peaks. Although designed for rapid LC separations, the integrated column–electrospray interface is also able to provide extended analyses of selected components using a technique known as "peak parking." (J Am Soc Mass Spectrom 1998, 9, 194–201) © 1998 American Society for Mass Spectrometry

The emerging field of "Proteome" research has been driven by the ability to rapidly identify proteins of biological interest following their electrophoretic separation/purification in an acrylamide gel medium. Elegant methods for their proteolytic digestion, by using in situ and Western blot methods, and subsequent extraction for mass spectral analysis have been described [1, 2] and widely practiced. The increased sensitivity of the new microelectrospray techniques [3, 4] for the analysis of these complex digest mixtures has reduced the sample requirements for protein identification to a fraction of the extract from a Coomassie blue-stained gel [5] or to a single silver-stained spot [6]. Automated methods of data reduction (protein identification) have been approached using peptide mass [2] and partial sequence information, or sequence tags, [7] and by correlation of experimentally derived CID spectra with synthetic spectra drawn from the appropriate databases [8]. The once difficult and time consuming task of protein identification has become "routine" by these combined improvements in sample handling, mass spectral analysis, and data interpretation. As the application of mass spectral methods for protein identification has expanded, increased sample throughput has become a principal area of focus for improving the methodology.

Matrix assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometric analysis of unseparated in-gel digest mixtures [9] has the capacity of for high sample throughput with per sample analysis times as short as 5–6 min [10]. However, when more than one protein is present in a gel band or the extraction yields analyses for only a few peptides, the MALDI-TOF data alone may not be sufficient for an unambiguous assignment. Analysis of peptide mixtures by liquid chromatography on-line with electrospray tandem mass spectrometry has the advantage of producing both molecular weight information for each peptide as well as sequence specific fragmentation in the form of collision induced dissociation (CID) spectra. With automated data controlled analysis procedures [11], both types of information can be collected in a single LC/MS run. Along with providing a temporal separation, microelectrospray LC/MS can produce a 10–20 fold increase in sensitivity due to the inherent concentrating power of reverse-phase chromatography [12]. The advantages of an LC/MS analysis generally come at the expense of sample throughput. In the initial description of our novel microcapillary LC system, the time compression benefits of the pressure programmed solvent delivery system were shown to decrease the total run time of a standard peptide map by 50% [13] and aid in the rapid re-equilibration of the reverse-

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phase column. The ability to selectively extend the analysis time under peaks of interest has more recently been demonstrated [4, 12] and termed "peak parking." The ability to manipulate both ends of the time spectrum (compression and expansion) which we term "variable flow chromatography" allows the needs of mass spectrometer to dictate its terms to the LC system without compromising chromatographic resolution [14].

We have now adapted the principals of variable flow chromatography to very rapid LC/MS analyses and applied it to the analysis and identification of gel separated proteins. Any rapid separation technique, whether it be packed liquid chromatography (LC), open tubular chromatography (OTC), capillary electrochromatography (CEC), or capillary electrophoresis (CE), presents an added challenge to the mass spectrometer used to perform the on-line analysis. As peak widths narrow in time, the mass spectrometer must be able collect spectra at a rate sufficient to keep pace with the chromatography. The scan rates for beam type mass spectrometers such as quadrupole and magnetic sector analyzers are generally insufficient for such analyses. Recently, Wu et al. reported the rapid separation and MS analysis of peptide mixtures in less than 6 min using capillary electrochromatography (CEC) coupled to custom built hybrid ion trap–time of flight mass spectrometer [15]. In this report, we describe how LC/MS analyses can be done in a similar time frame using an unmodified, commercially available quadrupole ion trap mass spectrometer.

Experimental

Materials and Suppliers

The fused silica capillary (FSC) was purchased from Polymicro Technologies (Phoenix, AZ). The Vydac C_{18} packing was purchased from The Separations Group (Hesperia, CA). The acetonitrile was from Burdick and Jackson (UV grade, Muskegon, MI) and the sequanal grade TFA was purchased from Pierce (Rockford, IL). The equine cytochrome *c* was from Sigma (St. Louis, MO). The one dimensional polyacrylamide gel bands were submitted to Dr. Kristine Swiderek (Protein Structure Core Facility, Division of Immunology, Beckman Research Institute of the City of Hope) by Dr. Steve Esworthy and Dr. Maria Mas, also of the Beckman Research Institute of the City of Hope. The methylated porcine trypsin used for the in situ digestion was from Promega (Madison, WI). All other reagents or buffers were of reagent grade or better.

Sample Preparation

A standardized Lys-C digest of equine cytochrome *c* was used to evaluate the chromatographic separation and to provide a quantitated sample for the sensitivity experiments. The digest was performed as previously described [16] and diluted before use in 1% acetic acid as necessary. The 1-D gel samples were submitted to the Protein Structure Core Facility at the City of Hope as single spots from a Coomassie blue-stained gel. The in situ digestion of the destained bands were performed by the method of Hellman as described previously [1, 5]. The final extraction volume was 20 μL.

Microcapillary HPLC

All LC/MS analyses were performed using an Apple Macintosh controlled microcapillary HPLC system developed at the City of Hope and previously described by the authors [13]. The standard gradient was from 2% to 92% Buffer B over 60 min using reduced TFA buffers (A, 0.02% TFA; B, 90% Acn, 0.014% TFA) at an intended flowrate of 200 nL/min. Rapid analyses were performed using the same gradient but at higher flowrates which effectively steepens the gradient by the same factor (6×). In general, sample injection was at 2000 lb/in.^{2} (~13–14 uL/min) for 90 s followed by the injection of the preformed gradient and a controlled reduction in system pressure to 200 lb/in.^{2} over 30 s. The running pressure remained at 200 lb/in.^{2} for the rapid analyses but was further reduced to 35 lb/in.^{2} over 6 s for the standard and variable flow experiments. Selected peak parking was initiated by reducing the system pressure to 10 lb/in.^{2}, venting and rezeroing the pressure transducer and closing the system without restarting it. The system flowrate was then governed by the combination of the gas pressure over the solvent reservoirs (3 lb/in.^{2}) and the electrospray induced flow as previously described [12].

Integrated On-Line Microspray Interface

The 150-μm-i.d. × 350-μm-o.d. on-line microspray needles used in these analyses were pulled using a commercial laser-based micropipette puller (Sutter Instrument, Novato, CA, model p-2000) to a terminal i.d. of approximately 5–10 μm. The programmed values for heat, filament, velocity, delay, and pull strength were 400, 0, 30, 125, and 0, respectively. The program cycled twice before separation occurred. The final assembly of the microspray needle was performed under a low power (6×) dissecting scope as previously described [4] except the second transfer line was withdrawn after the frit (Durapore) had been placed in its final position. The fritted tip was then coupled to a Valco 1/16 in. × 1/32 in. ZDV reducing union and packed at 4000 lb/in.^{2} using a Vydac, 5-μm, C_{18} packing as previously described for microcapillary columns [16]. The packed tip was connected to a 75-μm-i.d. × 350-μm-o.d. transfer line using a PEEK capillary Tee (Valco) and graphite ferrules. A 0.3-mm gold wire was introduced through the off-axis inlet to apply the electrospray potential. A 2–3-mm section of polyimide coating was removed from the 75-μm-i.d. transfer line to form an on-column flowcell to monitor sample injections and the progres-

sion of the organic gradient. The UV absorbance of the solvent stream was monitored at 200 nm as described previously [13].

LCQ ITMS–Microspray Interface

The microspray interface designed for the LCQ ITMS utilized a multiaxis translational positioner mounted on a custom, dove-tailed, Plexiglas platform in place of the original Finnigan source as previously described [12]. The two sources, standard and microspray, are interchangeable in a manner of minutes. Real time observation of the microspray tip was achieved using a dedicated CCD video system [12].

Mass Spectral Analysis

All mass spectral analyses were performed using a Finnigan LCQ ITMS equipped with a custom microspray interface. The LCQ was operated under manual control in the Tune Plus view with the Automatic Gain Control (AGC) active unless stated otherwise. The AGC targets were: Full MS—$5e+007$, MS^n—$2e+007$ and Zoom MS—$2.50e+006$. The default maximum injection time was 500 ms. The number of "micro scans" collected were: 3, 5, and 5 for full mass range, MS^2, and zoom scans, respectively, unless otherwise noted. Rapid peptide mapping experiments used a fixed ion injection time of 50 ms and a single microscan count. Rapid LC/MS/MS experiments were performed under the default AGC parameters with the exception of a single microscan in the FMS mode for rapid parent ion selection.

Data Analysis

The peptide masses obtained by the rapid zoom scan analysis of the cytochrome *c* digest were searched against the Owl.r29.4 protein database using the MS-Fit function of the PROTEINPROSPECTOR program [17] developed at the University of California, San Francisco and available via the internet at http://prospector.ucsf.edu. The search was limited to proteins <100,000 Da, the minimum number of peptides required for a match was set at 5, the maximum number of missed cleavages was set at 1. Modifications considered were N-terminal Gln to pyroGlu, oxidation of methionines, and acetylation of the aminoterminus of the protein. Three separate searches were performed with peptide mass tolerances of ±0.2, 0.5, and 1.0 Da.

The quality of the CID spectra collected at all flow rates were evaluated using the Sequest database search algorithm [8] as an objective standard. All peptide CID spectra were searched against the full Owl.fasta database under enzyme constraints (trypsin) with a 1.5-u parent and fragment mass tolerance.

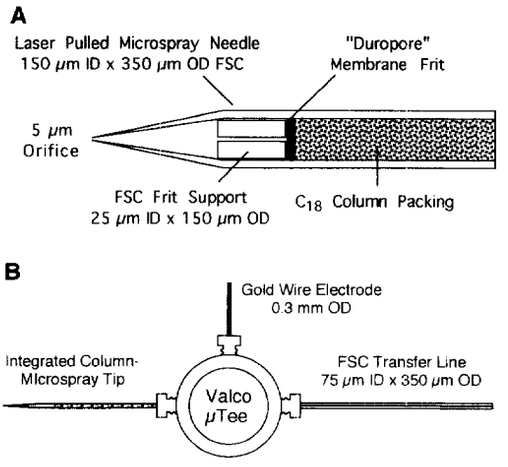

Figure 1. A high throughput, variable flow, microspray needle design featuring an integrated reverse-phase column (**A**) and a simplified, epoxy-free, solvent–electrode interface (**B**).

Results and Discussion

Electrospray Interface

The microscale electrospray interface utilized for rapid LC/MS analyses (Figure 1) is similar to the design of Emmett and Caprioli [18]. The needle is pulled from a length of 150-μm × 350-μm fused silica capillary (FSC) tubing using a CO_2 laser puller. A short piece of 25-μm × 150-μm FSC is used to support a membrane that serves as a frit for the column bed. The 5-μm needle orifice is large enough that packing the pulled tip is as easy as packing a conventional column. The inner piece of FSC tubing used to support the membrane filter introduces a small postcolumn dead volume. Although this may preclude its use for the attomole level analyses described by Emmett and Capriolli [18], it is quite adequate for low fmol peptide analyses. The pulled fused silica needles can also be packed without the membrane filter, but in our hands results have been inconsistent with frequent clogging of the orifice. The 150-μm column diameter was chosen because the 5-μm tips are easy to make reproducibly from this size tubing and very difficult to make using smaller diameter tubing.

The integrated tip is coupled to the LC system using a tee constructed from PEEK. The gradient from the LC pump is delivered to the tee using a 75-μ-m i.d. FSC transfer line. The third port of the tee is occupied by a 0.3-mm gold wire electrode for the application of the electrospray potential. The minor dead volume contained within the union is without consequence because it is at the head of the column. Tip replacement is facilitated through the use of "fingertight" fittings and graphite ferrules. As with all forms of capillary chromatography, column lifetime is sample dependent. With reasonable care not to inject large quantities and filtering of samples, a single column/tip was used for >40 injections over 2 days without affecting column

performance. With periodic trimming to remove accumulating debris from the head of the column, tip longevity can be greater than 40 h (1 week).

Because the integrated column/electrospray needle is assembled without the use of epoxy, it is free from the chemical background that would occur at low levels using our previous design. With the column in the needle, there is no possibility for UV detection of peaks eluting from the column. We have found it useful to incorporate a UV flow cell in the transfer line to monitor the refractive index change as the gradient is delivered to the column. Proper performance of the solvent delivery system is readily confirmed, and if the volume between the gradient valve and the flow cell is known, a reasonable value for the flow rate can be calculated from the delay between injection of the gradient and the response at the UV detector.

Optimization of Ion Trap Analysis Parameters for Rapid LC/MS Analyses

The reduced backpressure of the new column design made it possible to load samples at flow rates of 13–15 μL/min at 2000 lb/in.^{2}. Rapid gradient elutions could then be performed at 200 lb/in.^{2} (1.3–1.5 μL/min) to achieve complete baseline separation of the cytochrome *c* peptide digest mixture in less than 6 min although the mass spectral analysis was limited to a few scans per peak (data not shown). Reoptimization of the mass spectrometer parameters was required to take advantage of the rapid separation. The duty cycle of the LCQ is a function of the type of mass analysis desired (full mass range, zoom, or MS^n) the number of microscans collected for each data scan, and the available sample ions if the analysis is performed under automatic gain control (AGC). The rapid acquisition of full mass range spectra from 300 to 1850 m/z at a rate greater than 2 scans/s was achieved by reducing the micro scan count to one, disabling the AGC, and using a fixed ion injection time of 50 ms. Under these conditions, the rapid, sensitive, analysis of the peptide digest standard could be achieved using sample amounts ranging from 500 fmol (Figure 2A) to 10 fmol (Figure 2B). The average peak width increased from 3.4 s (8 scans) at 10 fmol to 4.2 s (9.5 scans) at 500 fmol. The additional peaks evident in the chromatogram for the 10-fmol sample are polymeric contaminants seemingly extracted from the polypropylene sample tube. The broadly eluting peak labeled with "o" was a contaminant (m/z 419) of unknown origin. As would be expected, ions in the spectra for the higher sample amount were more intense (Figure 2C,D). However, as a consequence of turning off the AGC and loading too many ions into the trap, there was also a significant shift (0.5 m/z) in the mass value for the doubly protonated molecule (m/z 736). Thus, although the increased duty cycle allows the mass spectrometer to keep pace with the rapid LC separation, the value of the full mass range spectra is compromised by the decreased accuracy of the mass values.

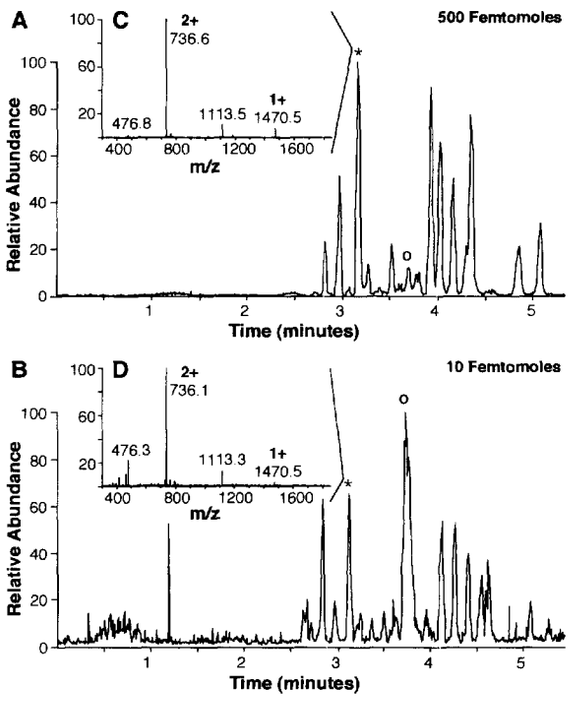

Figure 2. Rapid LC/MS analyses over a high dynamic range. Base peak chromatograms of the LC/MS analysis of the cyt. *c* standard digest at the 500 (**A**) and 10 fmol (**B**) level. Averaged spectra for the labeled peak (*) obtained from 500 (**C**) and 10 fmol (**D**) of the injected standard.

Valaskovic and McLafferty recently demonstrated low attomole sensitivity for CE separated proteins by optimizing the ion injection parameters of an FTICR mass spectrometer such that the entire peak was consumed in a single injection event [19]. A similar approach was taken with the LCQ ion trap. By adopting a mean peak width of 4 s and limiting the data-dependent analysis to a single event, the LCQ was programmed to collect a single, higher resolution zoom scan over peaks eluting from the column. The number of microscans collected for each zoom scan was set at 20 to ensure that the entire peak was consumed. Otherwise, the subsequent full mass range scan would initiate a second data-dependent analysis on the same base peak ion at the tail end of the peak resulting in zoom scans being collected when few or no ions were present. Automatic gain control with a 50-ms maximum injection time prevented putting too many ions in the trap. The full mass range microscan count was kept at one. Using these parameters, it was possible to obtain good zoom scans and accurate mass values for all of the major components in the standard digest mixture (Figure 3, Table 1). The zoom scan spectra given in Figure 3B,C are representative of the quality of the spectra for singly and doubly charged ions. Mass values given in Table 1 are accurate to ± 0.15 m/z. The accuracy of the peptide mass values can have a dramatic effect on the database

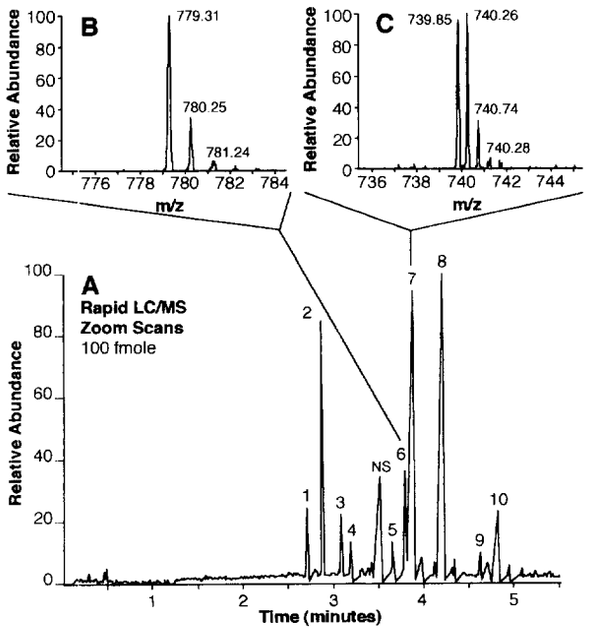

Figure 3. The rapid LC/MS zoom scan analysis of the standard digest mixture at the 100-fmol level. (**A**) Basepeak chromatogram of the cyt *c* standard digest. The peptide sequence and zoom scan derived mass values of the numbered peaks are given in Table 1. Representative zoom scan spectra for a singly charged ion (**B**, peptide 6) and a doubly charged ion (**C**, peptide 7) acquired in the rapid analysis. Each of these spectra is the average of 20 microscans collected in a single zoom scan event.

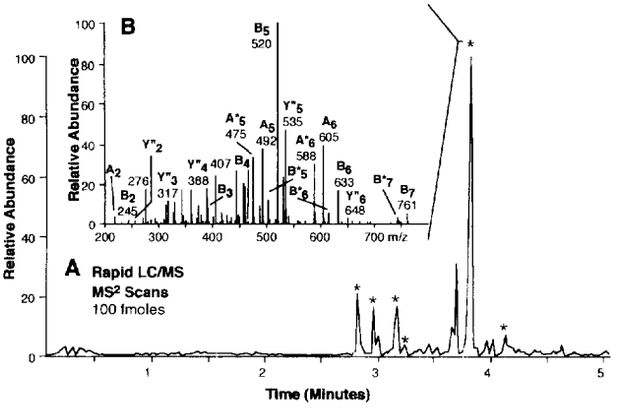

Figure 4. Rapid LC/MS/MS analyses of the standard test digest mixture performed at the 100 fmol level. (**A**) Basepeak chromatogram: The peaks labeled "*" were matched with the correct sequence with a Sequest search of the Owl.fasta database. (**B**) MS^2 spectra obtained from the major peptide.

search results. When the results from the standard digest mixture were searched using MS-Fit [17] against the database with a mass tolerance of 0.2 Da (see Experimental section for other search parameters), a match was made with 19 entries. When the mass tolerance was increased to 0.5 Da, the number of matches increased to 71 and at a mass tolerance of 1.0, there were 992 matches. The peptides listed in Table 1 represent a combined sequence coverage of 78.8%. A number of other peptides resulting from nonspecific cleavages were assigned based on their masses but not included in the table.

A similar "single-shot" approach was applied to the rapid LC/MS/MS analysis of a complex mixture. Because MS/MS analyses require higher numbers of ions than a zoom scan, the default injection time was raised to 500 ms. With the absence of a zoom scan to define the charge state, a default value of two was used to set the mass range for the MS^2 data collection. The microscan count remained at one for the full mass range scan and was set at five for the MS^2. The rapid LC/MS/MS analysis of the standard peptide digest at the 100 fmol (Figure 4) yielded CID spectra for a majority of the major components. The spectrum given in Figure 4B was representative of the quality of the data. A Sequest [8] search of the Owl.fasta database resulted in six spectra being matched to the correct peptide sequence (labeled "*" in Figure 4A). The same experiment at the 25 fmol level (data not shown) yielded three "direct" hits with a forth having the correct sequence ranked

Table 1. Rapid LC/MS – zoom scan analysis of the standard Endo Lys C digest of equine cytochrome *c* at the 100-fmol level. Peptide numbers refer to the labeled peaks in the base peak chromatogram in Figure 4. The combined mass measurements represents 78.8% of the protein sequence

			Monoisotopic mass		
Peptide	Residue	Sequence	Obs. *m/z*	Calc. *m/z*	Charge
1	9–13	IFVQK	634.26	634.39	1+
2	74–79	YIPGTK	678.23	678.38	1+
3	40–53	TGQAPGFTYTDANK	735.75	735.84	2+
4	56–60	GITWK	604.20	604.35	1+
5	28–39	TGPNLHGLFGRK	648.80	648.86	2+
6	80–86	MIFAGIK	779.31	779.45	1+
7	88–99	KTEREDLIAYLK	739.85	739.91	2+
8*	14–22	Heme + CAQCHTVEK	817.26	817.32	2+
9	61–72	EETLMEYLENPK	748.23	748.35	2+
10	56–72	GITWKEETLMEYLENPK	1041.01	1041.02	2+

* Not identifiable in a peptide mass dependent database search due to the post-translational addition of the prosthetic group.

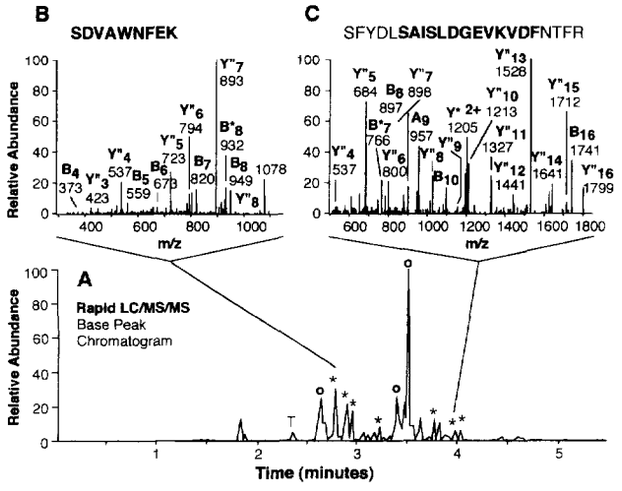

Figure 5. The rapid LC/MS/MS analysis of a 1-D in-gel digest mixture. (**A**) basepeak chromatogram. The peaks labeled "*" were correctly identified by a Sequest search of the Owl.fasta database, the peaks labeled "o" were correlated manually following the initial identification of the protein (human glutathione peroxidase). The trypsin peptide VATVSLPR (labeled "T") was also identified. Typical "single shot" MS/MS spectra obtained from a singly charged ion (**B**) and a doubly charged ion (**C**). Those portions of the peptide sequences that could be assigned based on the fragment ion spectra are in boldface.

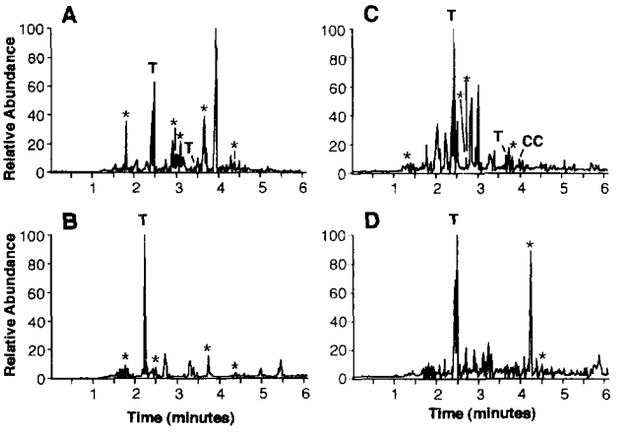

Figure 6. Rapid LC/MS/MS analysis of multiple in-gel digest mixtures under high-throughput conditions. The time between successive sample injections was 10 min. Peptide peaks labeled "*" were matched to a homologous chaperonin sequence by a Sequest search of the Owl.fasta database under enzyme constraints. The parent and fragment mass tolerance was 2 u. Trypsin autodigestion peptides are labeled "T" and a single cytochrome *c* peptide (carryover from the standard) is labeled "CC."

second. The narrower peak widths at the decreased sample level often left too few ions for the MS^2 analysis, particularly if the full mass range scan was collected sometime other than the beginning of the peak.

Rapid Identification of Gel Separated Proteins

To test the capability of the rapid LC/MS/MS method to identify peptides in more complex mixtures, the analysis of an in-gel digestion extract from a single Coomassie blue-stained band from a 1-D gel separation was performed (Figure 5). A 5% aliquot of the digest extract was sufficient to produce seven CID spectra (labeled "*" in Figure 5A) that were correlated to human glutathione peroxidase using the Sequest database search program. The total time required for the analysis was less than 5 min. An additional three peptides were confirmed manually once the protein had been identified (labeled "o," Figure 5A). A single peptide derived from trypsin was also identified (labeled "T," Figure 5A). Typical CID spectra for a 1+ ion and a 2+ ion are presented in Figure 5B and 5C, respectively.

A previously described feature of the variable flow LC system is the ability to rapidly equilibrate the column to initial conditions using the combination of an increased flow rate and the manual removal of the gradient from the solvent path of the LC [13]. By incorporating this strategy with the integrated microspray system, the duty cycle of the mass spectrometer could be increased to 6 samples/h using a 10-min injection cycle time. The base peak chromatograms for the sequential analysis of four in-gel digest mixtures in 40 min are shown in Figure 6. Each LC/MS/MS analysis was completed within 6 min and 4 min were allowed for returning the column to initial conditions. Samples were derived from the proteins that form the rabbit cytosolic chaperonin complex. The Sequest program was used to correlate the acquired spectra to known sequences contained in the Owl.fasta database. In this instance, the sequences for the rabbit chaparonin proteins are not contained in the database, but there is enough sequence identity with the mouse chaparonin proteins that all four proteins could be identified as being homologous to specific mouse chaparonin subunits. Peptide peaks for which a positive match was made by Sequest are labeled "*" in each of the four base peak chromatograms (Figure 6A–D). A major component of each of these analyses is the tryptic peptide VATVSLPR (labeled "T" in each chromatogram). The rapid LC/MS/MS analyses of these peptide digests of 50-kDa proteins represents a rigorous test of the ability to reliably screen complex tryptic digest mixtures to identify proteins using database searching.

Peak Parking

Although the emphasis in this work has been on coupling mass spectrometry with a high speed LC separation, it should also be pointed out that the same hardware components can be used for separations done on a longer time frame. Our previous microspray interface had been optimized for extended analyses via variable flow chromatography and "peak parking" [12, 20]. The ability to park on an LC peak and extend its' mass spectral analysis requires the LC system to depressurize the microspray tip before the desired component

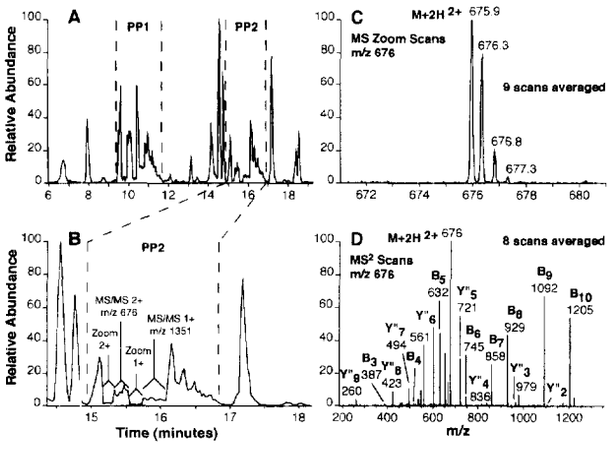

Figure 7. (**A**) The basepeak chromatogram of the LC/MS analysis of 100 fmol of the endo Lys C digest mixture of equine cytochrome *c* showing two peak parking events (PP1 and PP2). (**B**) The expanded basepeak chromatogram over PP2 featuring sequential zoom (narrow mass range, high resolution) scans and MS^2 analyses over the 1+ and 2+ ions of the peptide TEREDLIAYLK (MW = 1349.72). (**C**) Zoom scan and (**D**) MS^2 spectra obtained from the 2+ ion at m/z 676. The fragment ions are labeled using the nomenclature of Roepstorff and Fohlman [21].

has been expelled. The best results have typically been obtained from the smallest tips, 1–2 μm, which, unfortunately, are the easiest to clog. At the same time, it was important not to overtighten the fittings on the union connecting the tip to the electrode–transfer line assembly. Previous experience has shown that microspray needles as large as 4-μm i.d. are capable of limited peak parking [20].

By integrating the column within the tip, the negative influence of the union between the column and electrospray nozzle has been eliminated and a greater degree of time extension from a larger orifice has been achieved. This is illustrated by the extended analysis on two components of a standard peptide digest mixture (PP1 and PP2 in Figure 7A). The available analysis time for each component was extended approximately eight to tenfold compared to the peak widths of the neighboring peptides eluting under constant flow. Peaks observed during peak parking events PP1 and PP2 are the result of changing scan parameters and are not due to different components eluting from the column. During the second peak parking event (PP2, Figure 7B), there was sufficient time extension to manually collect high resolution, narrow mass range (zoom) scans, and MS^2 analyses on the 1+ and 2+ ions of each selected peptide under optimized CID parameters. Even after manual zoom scans (Figure 7C) and MS^2 scans (Figure 7D) of the 2+ ion at m/z 676, there was sufficient time remaining under the peak to consider the additional analysis of coeluting components.

The extension of the peak analysis time could have been longer had the pressure differential between the running (35 lb/in.^{2}) and parked (3 lb/in.^{2}) settings been greater. In previously reported work [12, 20], the usual running pressure was 150–250 lb/in.^{2} which provided greater flexibility in the length of time that data could be collected over a peak. However, reproducible peak parking performance is in general easier to obtain using the column within the tip design.

Conclusion

Rapid LC/MS analyses of complex peptide mixtures can be achieved using a microscale interface in which the reverse phase chromatography support is packed directly into a pulled fused silica electrospray needle. Using a pressure controlled solvent delivery system, the column can be equilibrated, the sample loaded, and the gradient delivered to the head of the column at a flow rate ten times that used for the separation. Under these conditions, reasonable separations of complex peptide mixtures can be accomplished in 6 min with 10-min total elapsed time between successive sample injections. A special set of analysis parameters for the Finnigan LCQ ion trap mass spectrometer have to be used in order to be compatible with the narrow peak widths of the eluted peptides. A duty cycle of 2 scans/s for broad mass range scans be achieved for sample amounts from 10 to 500 fmol by turning off the automatic gain control and using a fixed ion injection time for the trap. However, the observed mass values for intense doubly charged ions can be off by as much as 0.5 m/z. Alternatively, a data controlled approach can be used to obtain either accurate mass values or sequence information for rapidly eluting components. When a candidate ions is detected in the full mass range scan, the mass spectrometer is programmed to switch to collect either a narrow mass range, higher resolution (zoom) spectrum, or a CID fragment ion (MS^2) spectrum. Parameters are set such that the entire peak is used to collect the data. The identity of the protein can be assigned using existing database search programs that match either the set of accurate peptide masses obtained from a zoom scan spectra or the sequence information contained in the MS^2 spectra. This methodology provides the means to carry out high throughput screening of large numbers of samples with low femtomole sensitivity using a relatively inexpensive mass spectrometer. The same experimental setup is well suited for LC separations done within more conventional time frames when more comprehensive analysis of a complex mixture is required. Using the peak parking technique, the analysis time for selected components can be extended by as much as a factor of 10.

References

1. Hellman, U.; Wernstedt, C.; Gonez, J.; Heldin, C.-H. *Anal. Biochem.* **1995,** *224,* 451–455.
2. Henzel, W. J.; Billeci, T. M.; Stults, J. T.; Wong, S. C.; Grimley, C.; Watanabe, C. *Proc. Natl. Acad. Sci. USA* **1993,** *90,* 5011–5015.
3. Wilm, M.; Mann, M. *Anal. Chem.* **1996,** *68,* 1–8.

4. Davis, M. T.; Stahl, D. C.; Hefta, S. A.; Lee, T. D. *Anal. Chem.* **1995,** *67,* 4549–4556.
5. Blyn, L. B.; Swiderek, K. M.; Richards, O.; Stahl, D. C.; Semler, B. L.; Ehrenfeld, E. *Proc. Natl. Acad. Sci. USA* **1996,** *93,* 11115–11120.
6. Wilm, M.; Shevchenko, A.; Houthaeve, T.; Breit, S.; Schweigerer, L.; Fotsis, T.; Mann, M. *Nature* **1996,** *379,* 466–469.
7. Mann, M.; Wilm, M. *Anal. Chem.* **1994,** *66,* 4390–4399.
8. Eng, J. K.; Mccormack, A. L.; Yates, J. R. *J. Am. Soc. Mass Spectrom.* **1994,** *5,* 976–989.
9. Shevchenko, A.; Wilm, M.; Vorm, O.; Mann, M. *Anal. Chem.* **1996,** *68,* 850–858.
10. Shevchenko, A.; Mortensen, P.; Mann, M. *Biochem. Soc. Trans.* **1996,** *24,* 893–896.
11. Stahl, D. C.; Swiderek, K. M.; Davis, M. T.; Lee, T. D. *J. Am. Soc. Mass Spectrom.* **1996,** *7,* 532–540.
12. Davis, M. T.; Lee, T. D. *J. Am. Soc. Mass Spectrom.* **1997,** *8,* 1059–1069.
13. Davis, M. T.; Stahl, D. C.; Lee, T. D. *J. Am. Soc. Mass Spectrom.* **1995,** *6,* 571–577.
14. Lee, T. D.; Davis, M. T.; Stahl, D. C.; Swiderek, K. M.; Esworthy, R. S.; Hefta, S. A. *Proceedings of the Japanese Society for Biomedical Mass Spectrometry,* Vol. 21, pp. 17–32, 1996.
15. Wu, J.-T.; Huang, P.; Li, M. X.; Qian, M. G.; Lubman, D. M. *Anal. Chem.* **1997,** *69,* 320–326.
16. Davis, M. T.; Stahl, D. C.; Swiderek, K. M.; Lee, T. D. *Methods: A Companion to Methods in Enzymology* **1994,** *6,* 304–314.
17. Clauser, K.; Baker, P. *Protein Prospector,* V. 3.0, 1997, University of California, San Francisco, Available on the Internet at http://prospector.ucsf.edu.
18. Emmett, M. R.; Caprioli, R. M. *J. Am. Soc. Mass Spectrom.* **1994,** *5,* 605–613.
19. Valaskovic, G. A.; Kelleher, N. L.; Mclafferty, F. W. *Science* **1996,** *273,* 1199–1202.
20. Davis, M. T.; Lee, T. D. *44th Conference on Mass Spectrometry and Allied Topics,* Portland, OR, American Society of Mass Spectrometry, 1996; p 205.
21. Roepstorff, P.; Fohlman, J. *Biomed. Mass Spectrom.* **1984,** *11,* 601–602.